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Short communication

Selective and specific detection of sulfate-reducing bacteria using potentiometric stripping analysis

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ABSTRACT

A fast, sensitive and reliable potentiometric stripping analysis (PSA) is described for the selective detection of the marine pathogenic sulfate-reducing bacterium (SRB), *Desulforibrio caledoiensis*. The chemical and electrochemical parameters that exert influence on the deposition and stripping of lead ion, such as deposition potential, deposition time and pH value were carefully studied. The concentration of SRB was determined in acetate buffer solution (pH 5.2) under the optimized condition (deposition potential of −1.3 V, deposition time of 250 s, ionic strength of 0.2 mol L^{-1} and oxidant mercury (II) concentration of 40 mg L^{-1}). A linear relationship between the stripping response and the logarithm of the bacterial concentration was observed in the range of 2.3×10 to 2.3×10^7 cfu mL^{-1}. In addition, the potentiometric stripping technique gave a distinct response to the SRB, but had no obvious response to *Escherichia coli*. The measurement system has a potential for further applications and provides a facile and sample method for detection of pathogenic bacteria.

1. Introduction

Sulfate-reducing bacteria (SRB) are anaerobic microorganisms that use sulfate as a terminal electron acceptor, resulting in the production of sulfide. Sulfide is highly corrosive and toxic, thus it can be a serious problem for industries, economies, and ecological systems, such as the offshore oil industry. Rapid and sensitive approaches for SRB detection are essential for applications such as food safety, environmental monitoring, and clinical diagnosis, to allow faster decisions in dealing with public health issues such as food poisoning, water pollution or disease outbreaks. Various methods have been developed for the detection of SRB such as the most probable number (MPN) method involve a pre-enrichment step or a selective enrichment step followed by a biochemical test [1–3], the enzyme-linked immunosorbent assay for extra enzyme-label antibodies [4–6], and pertinent molecular techniques such as polymerase chain reaction [7,8] or fluorescence in situ hybridization [9]. Although these techniques for pathogen detection are sufficiently sensitive and selective, most have several disadvantages including time-consuming, cost-intensive, or technically complex.

Electrochemical techniques have emerged as extremely useful tools for pathogen detection in last decades. Various sensitive, reliable, and more rapid methods have been reported for determination and monitoring of microorganisms, including electrochemical impedance spectroscopy [10–12], differential pulse voltammetry [13], chronocoulometry [14], cyclic voltammetry [15] and electrical conductivity [16]. Among the electrochemical detection platform, as a sensitive and reliable electrochemical tool, stripping analysis has been applied in wide-ranging areas. The stripping analysis measurement has always been recognized as a powerful tool for measuring metal ions. This technique shows several advantages including low cost, simple operation, high sensitivity and excellent selectivity [17–19]. It occurs because in stripping analysis, an accumulation step is firstly performed to enhance sensitivity and selectivity. Compared to the stripping voltammetry, the stripping time is the signal measured in potentiometric stripping analysis (PSA) with higher accuracy and precision than the stripping current obtained from the stripping voltammetry. This is due that the time is the physical parameter measured in PSA that can be measured with higher accuracy, precision and resolution than currents used in the stripping voltammetric methods [20]. PSA technique have been successfully applied to metal ion determination such as zinc, cadmium and lead in a variety of medium including environmental water analysis [21–24], quality control of pharmaceutical formulation [25], determination of heavy metal in food industry [26–30], diagnosis of medical treatment and health [31,32]. In recent years, a new nanoparticle-based electrical

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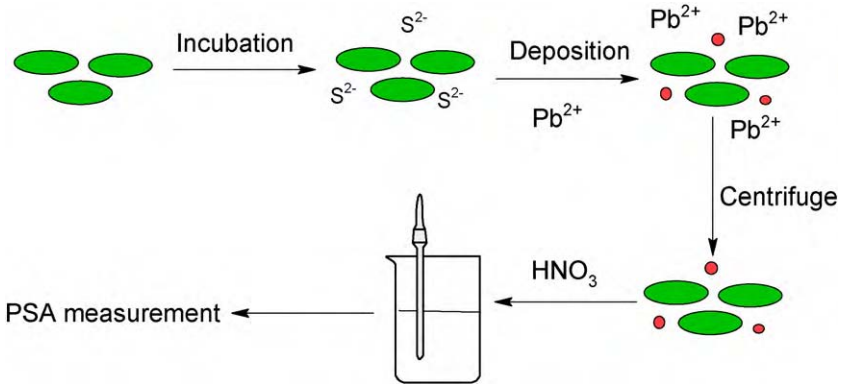

Fig. 1. The flow diagram of PSA measurement for detection of SRB.

detection of DNA hybridization, based on electrochemical stripping analysis of the colloidal gold tag, is presented according to Wang's paper [33,34].

In this work, the development of a reliable potentiometric stripping method based on the metabolic product of SRB, sulfide, for facile and rapid detection of SRB has been illustrated. With this method detection and determination of SRB can be achieved with high specificity and sensitivity.

2. Materials and methods

2.1. Chemicals

Mercury stock solution (40 mg L^{-1}) was prepared from their acetate salt without further purification. Acetate buffer solution (0.2 mol L^{-1}) was prepared without further purification from acetic acid and pH was adjusted with solution of concentrated NaOH. $Pb(CH_3COO)_2$ was also supplied by Sinopharm Chemical Reagent Co., Ltd., as were the analytical grade $MgSO_4$, NH_4Cl, Na_2SO_4, $CaCl_2$, Na_2HPO_4, NaOH, sodium lactate, and yeast extract used to prepare the modified Postgate's medium. The Postgate's medium was filtered with a 0.2 μm pore size filter. All other chemicals were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and Milli-Q water (TGI Pure Water Systems, USA) was used throughout.

2.2. Electrochemical measurements

Potentiometric stripping analysis (PSA) was carried out using a CHI760C (CH Instruments, Inc.) conventional three electrode system that included a platinum wire and an Ag/AgCl, 3 M KCl as the counter electrode and a reference electrode, respectively. Before starting a set of analyses, the glassy carbon electrode which was the GC electrodes were polished on a polishing cloth (CHI Inc.) with subsequently smaller particle (1.0 and 0.05 mm) of Al_2O_3 slurry, then rinsed with water, ultrasonicated in ethanol and doubly distilled water, respectively. Aliquots of stock solution of lead were added into the electrochemical cell containing 0.2 M acetate buffer solution (pH 5.2) and the Hg (II) ions at 40 mg L^{-1}. In these experiments, the potential was first held at −1.3 V for 250 s to electrochemically deposited lead onto the modified working electrode surface. All electrochemical measurements were made under stirring during the plating and accumulation steps. After a 60 s rest the electrochemical stripping was carried out from −1.3 V to −0.2 V with stripping current, 0 A. During the stripping step the solution was maintained under quiescent condition. The reproducibility of the stripping responses is represented by the error bar, which was obtained from the standard deviation for the three replicates.

2.3. Sample preparation

Before PSA measurement, the SRB samples needed to be prepared as shown in Fig. 1. A series of SRB culture (10 mL) from 10 to 10^7 cfu mL^{-1} were incubated at 30 °C for 8 h to produce the metabolic product, sulfide. Subsequently, 1 mL SRB culture was added into 0.2 mL 5 mmol L^{-1} Pb^{2+} solution for 1 h to deposit the sulfide. The PbS nanoparticles generated above were rinsed with ethanol three times and then resuspended in 1 M nitric acid (0.2 mL) under sonication for 2 h. The mixture was added into the electrochemical cell to perform potentiometric stripping measurement. All glassware were used for sample preparation and solution storage were decontaminated with 1.0 M nitric acid for 2 h. The digested samples were stored in polyethylene vessels at 4 °C.

2.4. Bacterial cultivation

The SRB seeds were isolated from marine mud collected from the Bohai Sea, China. A small amount of water is added and mixed with mud, and crushed to pass through a 2-mm-mesh-size screen. We used inoculating loop and aseptic technique, streak plate on the four quarter of the plate, crossing over the initial streak area four times under anaerobic condition. We chose individual colonies by lightly touching the top of an individual colony with a sterile loop or needle and streak on Postgate's medium under nitrogen-saturated condition. After the pure SRB culture was grown in modified Postgate's medium (1 L) containing 2 g $MgSO_4$, 1 g NH_4Cl, 0.5 g Na_2SO_4, 0.1 g $CaCl_2$, 0.5 g Na_2HPO_4, 2 mL sodium lactate, and 7.5 g yeast extract at 30 °C for 4 days, the bacterial cells were then isolated through centrifugation (4000 rpm, 15 min) and rinsed with phosphate buffer solution (PBS, pH 7.4) including 0.038 mol L^{-1} NaH_2PO_4 and 0.162 mol L^{-1} Na_2HPO_4 in three times. Before incubation, SRB samples were stored in the refrigerator at 4 °C for slow growth. The culture was serially diluted with physiological saline solution, and viable cell number was determined by the most probable number (MPN) method according to the American Society of Testing and Materials Standard D4412-84. The MPN method, otherwise known as the method of Poisson zeroes, is a method of getting quantitative data on concentration of discrete items from positive data. To increase the statistical accuracy of this test, standard MPN procedure uses a minimum of five tubes per dilution. After incubation, the pattern of positive tubes is noted, and a standardized MPN table is consulted to determine the most probable number of microorganisms per unit volume of the original sample. The Gram-negative bacterium, *Escherichia coli*, was from the contribution of Dr. Song Qin used as a control experiment.

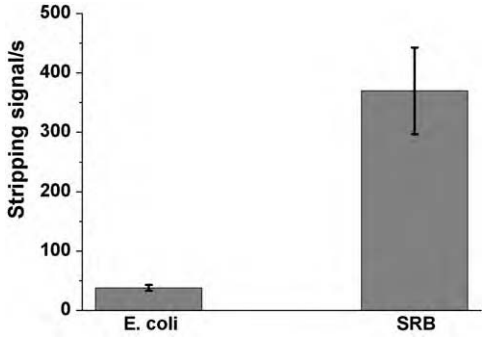

Fig. 2. PSA technique specificity for the detection of SRB. The concentration of SRB was 2.3×10^7 cfu mL^{-1}. The concentration of *E. coli* was 2.2×10^7 cfu mL^{-1}.

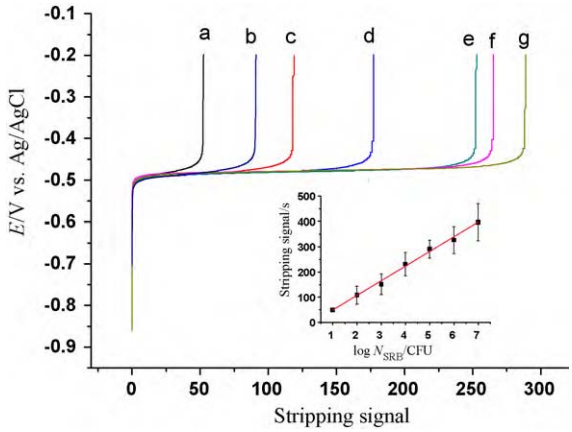

Fig. 3. PSA measurements obtained on SRB digested mixture after incubated for 8 h. Curves a–g represent SRB concentrations from 2.3×10 to 2.3×10^7 cfu mL^{-1}, respectively. The inset: concentration of SRB (log cfu mL^{-1}) versus the stripping signal values.

3. Results and discussion

3.1. Optimization of experimental parameters

Some experimental parameters were carried out to obtain the best optimize value for the PSA measurement. The assay conditions were optimized as a function of three parameters; namely, the electrodeposition potential and time and the effect of pH on the accumulation processes in the PSA measurement and the best optimize value were selected from the following experiments.

The pH could also contribute to the stripping signal change. The influence of pH on the stripping response was investigated in 0.2 M HAc–NaAc to optimize the assay conditions. The stripping response increased when the pH increased from 3.2 to 5.2 but then decreased at pH > 5.2. It is also interesting to note that the analytical signal significantly decreased at pH 5.2, which was due to hydrolysis of metal ions. Therefore, the optimized pH level of 5.2 was used in our study for the detection of SRB.

It is well known that the application of adequate electrodeposition potential in stripping analysis is very important to achieve the best sensitivity and selectivity. Thus, the influence of this parameter on the analytical response is shown. When an electrodeposition potential more negative than −1.3 V was used, an minor variation on the stripping signal was observed for lead. This is due that the electrodeposition potential is more negative than −1.3 V, an instable and uneven mercury film fabricated is unfavorable to the metal-amalgam formation [21]. However, the stripping signal decreased markedly over from −1.3 to −0.7 V because the low potential result in the difficult of metal reduction. Therefore, the optimized electrodeposition potential of −1.3 V was used in our study for the detection of SRB.

The electrodeposition time was studied over a range of times between 100 and 300 s. The result illustrates the dependence of the electrodeposition time and stripping response when a fixed lead ion concentration (0.5 mmol L^{-1}) was maintained. As the electrodeposition time increased, the increase in stripping response was rapid and almost linear at first, but then levelled off until a relatively stable platform was reached after 250 s. Hence, a further deposition time increase beyond 250 s practically does not improve the analytical signal. An electrodeposition time of 250 s was selected to effectively obtain the optimized signal, since no distinct changes in the stripping signal were observed at longer incubation times.

3.2. Specificity of assay

Specificity is one of the most important criteria for evaluating biosensor. In this work, the selectivity of the PSA technique against other bacteria was evaluated by measuring the change of stripping signal at the similar concentration. Fig. 2 shows the PSA measurement specificity for the detection of SRB. In this measurement, the stripping signal values increased less than 40 ± 5 s when *E. coli* were analyzed, whereas a distinct increase (383 ± 65 s) was obtained after incubating with SRB, indicating that the observed changes of the stripping signal with SRB were specific metabolic product, sulfide, which made this PSA measurement feasible for the detection of SRB.

3.3. Detection of SRB

Fig. 3 shows the results of PSA measurements obtained on the GC electrode with metal-amalgam film. The stripping time of the PSA plots increased regularly with the increase of the SRB concentration, indicating that SRB. This is because the metabolic product of SRB, sulfide, deposited more lead ion with increasing the concentration of bacteria. Seven SRB concentrations from 2.3×10 to 2.3×10^7 cfu mL^{-1} were prepared by serial dilution in PBS. As seen in the inset of Fig. 3, the results for the PSA assay show that concentration of bacteria and stripping response were highly correlated. A linear relationship between stripping response and the logarithm of the bacteria concentration was obtained for the concentration range from 2.3×10^2 to -2.3×10^7 cfu mL^{-1}, with a slope of 58.1 and a correlation coefficient of 0.997.

Various methods have been developed for the detection of SRB, including MPN [1–3], biochemical tests [4–6], and polymerase chain reaction [7–9]. Although these techniques for pathogen detection are sufficiently sensitive and selective, most have several disadvantages including being time-consuming (e.g., MPN), cost-intensive (e.g., specific enzyme-labelled antibodies), or technically complex (e.g., DNA analysis). Recently, our group has worked in the research line of the impedimetric immunosensor based on self-assembled monolayer immobilized with lectin and antibody for the detection of bacterial cell and the monitoring of SRB population [10,12]. Compared with methods for the detection of SRB, the typical impedimetric sensor based on self-assembled monolayer requires extensive and prolonged modification processes (more than 12 h), unlike the PSA technique, which can be used directly to detect sulfide or SRB in real matrix (less than 30 min).

We used the standard method for detection of sulfide produced from SRB to detect the recovery of the procedures. In detail, a series of SRB culture (10 mL) from 10 to 10^7 cfu mL^{-1} were incubated at 30 °C for 8 h to produce the metabolic product, sulfide. The iodometric determination of sulfide produced from SRB after incubated in 8 h including two reactions as follows. The main oxidation reaction (1): $S^{2-} + I_2 \rightarrow S + 2I^-$; and side reaction of sulfide (2): $S^{2-} + 4I_2 + 8OH^- \rightarrow SO_4{}^{2-} + 8I^- + 4H_2O$. The result of concentration

Table 1
Sulfide concentration of produced from a series concentration of SRB after incubation.

The concentration of SRB (cfu mL^{-1})	2.3×10	2.3×10^2	2.3×10^3	2.3×10^4	2.3×10^5	2.3×10^6	2.3×10^7
Sulfide concentration (mmol L^{-1})	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.10 ± 0.02	0.12 ± 0.02	0.21 ± 0.05	0.63 ± 0.16

of sulfide was shown in Table 1. Table 1 shows that the sulfide concentration increased with the bacteria concentration from 2.3×10 to 2.3×10^7 cfu mL^{-1}.

The real samples, which were treated according to Section 2.2, were resuspended with PBS buffer solution and then examined with the proposed PSA measurement. The technique designed in this study was used to monitor the variation in a microbial SRB population that was isolated from marine mud. The microbial population increased rapidly from 1.5×10^3 to 2.3×10^7 cfu mL^{-1} in 1–5 days in the growth phase. This trend displays a typical bacterial growth process. In addition, the MPN method was used as a control experiment to monitor the growth of the SRB population; the obtained growth curve was similar to that measured with the PSA analysis. These results indicated that the presented method was in acceptable agreement with the traditional method. Therefore, the proposed technique could be satisfactorily applied to the monitoring of SRB in growth processes.

4. Conclusion

We have described a faster and reliable method suitable for SRB detection based on the amplification of response of preconcentration in PSA measurement. The results indicate that the PSA technique yielded a distinct response to, SRB, but had no obvious response to Gram-negative bacteria, *E. coli*. Meanwhile, the optimal assays show that the method can obtain a supreme response while the electrodeposition potential and time in accumulation of measurement processes are −1.3 V and 250 s, respectively, under pH 5.2 PBS. A linear relationship between stripping response and the logarithm of the bacterial concentration was observed in the range 2.3×10 to 2.3×10^7 cfu mL^{-1}. To the best of our knowledge, this paper provides the first example of the use of PSA for directly detecting the microorganism.

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